

# METHODS OF MAKING ANTIBODY HEAVY AND LIGHT CHAINS HAVING SPECIFICITY FOR A DESIRED ANTIGEN

This is a continuation of application(s) Ser. No. 07/205,419 filed on 10 Jun. 1988, now U.S. Pat. No. 6,331,415, issued on 18 Dec. 2001, which is a continuation of Ser. No. 06/483,457 filed on 8 Apr. 1983, now U.S. Pat. No. 4,816,567, issued on 28 Mar. 1989, which applications are incorporated herein by reference and to which application(s) priority is claimed under 35 USC §120.

## BACKGROUND OF THE INVENTION

This invention relates to the field of immunoglobulin production and to modification of naturally occurring immunoglobulin amino acid sequences. Specifically, the invention relates to using recombinant techniques to produce both immunoglobulins which are analogous to those normally found in vertebrate systems and to take advantage of these gene modification techniques to construct chimeric or other modified forms.

### A. Immunoglobulins and Antibodies

Antibodies are specific immunoglobulin polypeptides produced by the vertebrate immune system in response to challenge by foreign proteins, glycoproteins, cells, or other antigenic foreign substances. The sequence of events which permits the organism to overcome invasion by foreign cells or to rid the system of foreign substances is at least partially understood. An important part of this process is the manufacture of antibodies which bind specifically to a particular foreign substance. The binding specificity of such polypeptides to a particular antigen is highly refined, and the multitude of specificities capable of being generated by the individual vertebrate is remarkable in its complexity and variability. Thousands of antigens are capable of eliciting responses, each almost exclusively directed to the particular antigen which elicited it.

Immunoglobulins include both antibodies, as above described, and analogous protein substances which lack antigen specificity. The latter are produced at low levels by the lymph system and in increased levels by myelomas.

#### A.1 Source and Utility

Two major sources of vertebrate antibodies are presently utilized—generation in situ by the mammalian B lymphocytes and in cell culture by B-cell hybrids. Antibodies are made in situ as a result of the differentiation of immature B lymphocytes into plasma cells, which occurs in response to stimulation by specific antigens. In the undifferentiated B cell, the portions of DNA coding for the various regions on the immunoglobulin chains are separated in the genomic DNA. The sequences are reassembled sequentially prior to transcription. A review of this process has been given by Gough, *Trends in Biochem Sci*, 6: 203 (1981). The resulting rearranged genome is capable of expression in the mature B lymphocyte to produce the desired antibody. Even when only a single antigen is introduced into the sphere of the immune system for a particular mammal, however, a uniform population of antibodies does not result. The in situ immune response to any particular antigen is defined by the mosaic of responses to the various determinants which are present on the antigen. Each subset of homologous antibody is contributed by a single population of B cells—hence in situ generation of antibodies is “polyclonal”.

This limited but inherent heterogeneity has been overcome in numerous particular cases by use of hybridoma technology to create “monoclonal” antibodies (Kohler, et al., *Eur. J.*

*Immunol.*, 6: 511 (1976)). In this process, splenocytes or lymphocytes from a mammal which has been injected with antigen are fused with a tumor cell line, thus producing hybrid cells or “hybridomas” which are both immortal and capable of producing the genetically coded antibody of the B cell. The hybrids thus formed are segregated into single genetic strains by selection, dilution, and regrowth, and each strain thus represents a single genetic line. They therefore produce immunoreactive antibodies against a desired antigen which are assured to be homogenous, and which antibodies, referencing their pure genetic parentage, are called “monoclonal”. Hybridoma technology has to this time been focused largely on the fusion of murine lines, but human-human hybridomas (Olsson, L. et al., *Proc. Natl. Acad. Sci. (USA)*, 77: 5429 (1980)); human-murine hybridomas (Schlom, J., et al. (ibid) 77: 6841 (1980)) and several other xenogenic hybrid combinations have been prepared as well. Alternatively, primary, antibody producing, B cells have been immortalized in vitro by transformation with viral DNA.

Polyclonal, or, much more preferably, monoclonal, antibodies have a variety of useful properties similar to those of the present invention. For example, they can be used as specific immunoprecipitating reagents to detect the presence of the antigen which elicited the initial processing of the B cell genome by coupling this antigen-antibody reaction with suitable detection techniques such as labeling with radioisotopes or with enzymes capable of assay (RIA, EMIT, and ELISA). Antibodies are thus the foundation of immuno diagnostic tests for many antigenic substances. In another important use, antibodies can be directly injected into subjects suffering from an attack by a substance or organism containing the antigen in question to combat this attack. This process is currently in its experimental stages, but its potential is clearly seen. Third, whole body diagnosis and treatment is made possible because injected antibodies are directed to specific target disease tissues, and thus can be used either to determine the presence of the disease by carrying with them a suitable label, or to attack the diseased tissue by carrying a suitable drug.

Monoclonal antibodies produced by hybridomas, while theoretically effective as suggested above and clearly preferable to polyclonal antibodies because of their specificity, suffer from certain disadvantages. First, they tend to be contaminated with other proteins and cellular materials of hybridoma, (and, therefore, mammalian) origin. These cells contain additional materials, notably nucleic acid fragments, but protein fragments as well, which are capable of enhancing, causing, or mediating carcinogenic responses. Second, hybridoma lines producing monoclonal antibodies tend to be unstable and may alter the structure of antibody produced or stop producing antibody altogether (Kohler, G., et al., *Proc. Natl. Acad. Sci (USA)* 77: 2197 (1980); Morrison, S. L., *J. Immunol.* 123: 793 (1979)). The cell line genome appears to alter itself in response to stimuli whose nature is not currently known, and this alteration may result in production of incorrect sequences. Third, both hybridoma and B cells inevitably produce certain antibodies in glycosylated form (Melchers, F., *Biochemistry*, 10: 653 (1971)) which, under some circumstances, may be undesirable. Fourth, production of both monoclonal and polyclonal antibodies is relatively expensive. Fifth, and perhaps most important, production by current techniques (either by hybridoma or by B cell response) does not permit manipulation of the genome so as to produce antibodies with more effective design components than those normally elicited in response to antigens from the mature B cell in situ. The antibodies of the present invention do not